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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Kevin G. Pinney et al.		
APPLICATION NUMBER:	**10/070,484**	EXAMINER:	Charanjit S. Aulakh
FILING DATE:	October 11, 2002	ART UNIT:	1625
FOR:	INDOLE-CONTAINING AND COMBRETASTATIN-RELATED ANTI-MITOTIC AND ANTI-TUBULIN POLYMERIZATION AGENTS		

March 9, 2004
Boston, Massachusetts

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. §1.131

I, Kevin G. Pinney, of Baylor University in Waco, Texas, declare and state that:

1. I am making this Declaration as a listed Inventor in the above-identified application ("the Application"). I am a synthetic organic chemist and have conducted research in this field for 21 years. I received a Doctorate in Chemistry in 1990 from the University of Illinois at Champaign-Urbana and am presently an Associate Professor in the Department of Chemistry and Biochemistry at Baylor University where I conduct research in drug discovery.

2. I am aware that in the Office Action dated September 10, 2003, the Examiner has cited Compound XVII (3-(3',4',5'-trimethoxybenzoyl)-2-(4"-methoxy-5"-hydroxyphenyl)-6-methoxylindole) and Compound XVIII (indole sodium phosphate prodrug), (collectively, the "Compounds") of Pero U.S. Patent No. 6,538,038 ("Pero") under 35 U.S.C. §102(e) as anticipating claims 1-6, 17, 19, 22, 24 and 41-47 of the Application. This declaration is being made to establish my invention of the Compounds under 37 C.F.R. 1.131(b) at a date prior to February 16, 2000, the effective filing date of Pero.

3. I am the inventor of the subject matter of claims 1-6, 17, 19, 22, 24 and 41-47 and hereby declare that I conceived of the invention claimed in at least these claims in the United States before February 16, 2000, the effective filing date of the cited subject matter in Pero. Furthermore, I diligently sought to reduce my invention to practice from prior to February 16, 2000 and up until September 15, 2000, at which time I constructively reduced my invention to practice by filing International PCT Application No. PCT/US00/25408 (hereinafter the "PCT Application"), to which the current Application claims priority. I continued to diligently pursue actual reduction to practice of the invention from September 15, 2000 until such time as it could be conclusively demonstrated that Compounds XVII and XVIII were successfully synthesized. A copy of the claims submitted in the PCT Application, and which claims are currently pending in the subject Application, is attached hereto as Exhibit 1. Note also that a copy of the claims as amended in the accompanying Response to Office Action is attached hereto as Exhibit 2.

4. In May 1999, a graduate student in my laboratory, Feng Wang, submitted a Master of Science thesis to the Faculty of Baylor University entitled "Stereoselective Synthesis of Conjugated Diene Systems and Design of New Tubulin Polymerization Inhibitors as Antimitotic Agents". This thesis summarized work that Wang had performed in my laboratory and under my direction, supervision, and advisement during the period of May 1996 to May 1999. Copies of the title page, abstract, and pages 72-75 and 108-109 of the thesis are provided in Exhibit 3.

5. In the second paragraph of the thesis abstract, Wang reported the synthesis of an "indole-based ligand", 3-(3',4',5'-trimethoxybenzoyl)-2-(4'-methoxyphenyl)-6-methoxyindole, which was therein designated Compound 33. To conclusively demonstrate that Compound 33 had indeed been synthesized, Wang provided ^{1}H and ^{13}C Nuclear Magnetic Resonance ("NMR") spectra on thesis pages 108 and 109 respectively. Wang went on to report in the third paragraph of thesis page 73 that Compound 33 displayed remarkable cytotoxicity against selected human cancer cell lines, as well as excellent tubulin inhibition.

6. Based on the promising activity of Compound 33, I proposed the synthesis of several of its analogs (see page 75 of Exhibit 3). One of the candidate analogs was the compound 3-(3',4',5'-trimethoxybenzoyl)-2-(4'-methoxy, 5'-hydroxyphenyl)-6-methoxyindole. Its structure is provided in the center of Figure 20 on page 75. 3-(3',4',5'-trimethoxybenzoyl)-2-(4'-methoxy, 5'-hydroxyphenyl)-6-methoxyindole is the same compound identified as Compound XVII by Pero.

7. At the time of my invention, I recognized the importance of using water-soluble phosphate ester derivatives of hydroxylated indole compounds. These phosphate esters impart improved solubility to the indole compound. Additionally, they would act as Prodrugs, which are inactive derivatives that are selectively dephosphorylated in the body to generate the active, tubulin-binding version of the indole compound. Most importantly, I recognized that indole phosphate ester prodrugs have the potential to cause destruction of tumor vasculature and improved tumor growth control. Having recognized the value of indole phosphate ester prodrugs, I proposed the chemical phosphorylation of Compound XVII to obtain Compound XVIII, an indole monophosphate prodrug.

8. On September 17, 1999, provisional patent application No. 60/154,639 (hereinafter, the "Provisional") was filed in the US Patent and Trademark Office. The Provisional provided a detailed description of the synthesis of Compound 33, its tubulin binding activity, and its cytotoxicity towards tumor cells. Claims were also submitted as part of the Provisional describing a group of trimethoxyphenyl-substituted indole ligands that are substituted further with one or more hydroxyl functional groups. The Provisional application also stated that a phosphate ester is an important molecular feature for targeting by selective dephosphorylation at sites of enhanced vascularization such as a tumor.

9. Beginning as early as January 2000, I began to enlist the technical assistance of students in my laboratory for projects related to the synthesis of the Compounds. A copy of my

discussion notes from this time period are provided in Exhibit 4. On page 2 of Exhibit 4 are illustrated five (5) compounds which I proposed for synthesis, two of which are Compounds XVII and XVIII. In the upper left hand corner was illustrated Intermediate #1, the TBS-protected, 2-bromo-3-hydroxy-4-methoxyacetophenone, which was a required precursor for the synthesis of the desired Compounds.

10. From the period of June 1, 1999 and throughout the period of my reduction to practice of the invention, I obtained research funding under a grant from OXiGENE, Inc., the assignee of the Pero patent. I submitted a supplemental grant application to OXiGENE, Inc. in order to secure additional funding for synthesis of the Compounds. A copy of the grant application dated prior to February 16, 2000 is provided in Exhibit 5. The grant application outlined a project proposal for the synthesis of a three candidate "Tumor Vascular Targeting Agents", one of which is Compound XVIII (see Figure 1, Page 1 of Exhibit 5).

11. As part of the above grant application, I provided a detailed synthetic route for the synthesis of Compound XVIII in the form of Scheme III (see Exhibit 5, Page 5). Scheme III outlined the following four major steps which are also recited in Figures 12, 13, and 14 of the subject Application:

    Step a) Preparation of a TBS-protected, 2-bromo-3-hydroxy-4-methoxyacetophenone ("Intermediate #1").

    Step b) Preparation of a TBS-protected 2-(3-hydroxy, 4-methoxyphenyl)indole ("Intermediate #2") by: i) treatment of *m*-anisidine and TBS-protected 2-bromo-3-hydroxy-4-methoxyacetophenone under basic conditions, ii) treatment of the resultant secondary amine with polyphosphoric acid (PPA), and iii) reprotection of the 3'-hydroxyl substituent.

    Step c) Preparation of Compound XVII by Friedel-Crafts acylation of the 2-phenylindole with 3,4,5-trimethoxybenzoyl chloride in the presence of 1,2-dichlorobenzene.

Step d) Preparation of Compound XVIII by: i) phosphorylation of Compound XVII with *in situ*-generated dibenzyl chlorophosphite, ii) cleavage of the resultant benzyl ester with trimethylchlorosilane/sodium iodide, and iii) treatment with sodium methoxide.

12. Steps a) through d) are based on methods well known to those skilled in the art of synthetic organic chemistry. Step a) is a modification of a well-known method for the production of halogenated acetophenones using a benzaldehyde as starting material. Steps b) through c) are slight modifications of the procedure outlined in the Provisional for the synthesis of Compound 33 (see Example 1, pages 15-17). Step d) is a minor modification of the procedure previously outlined by Pettit for the synthesis of Combretastatin A-4 Prodrug (*Anti-Cancer Drug Design*, 1998, 13: 183-191)

13. Select pages from the laboratory notebook of Heather O'Dell are provided in Exhibit 6. O'Dell performed several experiments in my laboratory and under my supervision and advisement that were directed towards some of the initial attempts at synthesizing the Compounds using the procedure described in Scheme III (see Exhibit 5, Page 5). In notebook entries dated January 13 and January 28, 2000, attempts to synthesize Intermediate #2 are recited, using m-anisidine and Intermediate #1 as reagents (see pages 1-2, Exhibit 6). These attempts were unsuccessful, and additional amounts of Intermediate #1 were required for repeat attempts to synthesis Intermediate #2. Additional notebook entries denote attempts to resynthesize adequate amounts of Intermediate #1 up until May 30, 2000 (see pages 3-8, Exhibit 6). Unfortunately, it could not be conclusively demonstrated that these attempts were successful in generating Intermediate #1.

14. Select pages from the laboratory notebook of Mallinath Hadimani are provided in Exhibit 7. Hadimani performed several experiments under my supervision and in my laboratory that were directed towards the synthesis of the Compounds. In a notebook entry dated June 18th, 2000, experimental procedures are recited for the synthesis of Intermediate #1, using

Isovanillin ("mbh-II") and TBSCl as starting materials. In a notebook entry dated June 19th, 2000, the successful synthesis of a TBS-protected Isovanillin ("mbh-II-1"), step 1 in the synthesis of Intermediate #1, was reported (see NMR of mbh-II-1 on page 2 of Exhibit 7). Additional entries on page 3-4 of Exhibit 7 report the successful treatment of mbh-II-1 with methyllithium to obtain the TBS-protected secondary alcohol, mbh-II-2 (see NMR of mbh-II-2 on page 4, Exhibit 7). In notebook entries dated June 20th and June 21st, 2000, Hadimani reported the successful oxidation of mbh-II-2 to provide the TBS-protected ketone (therein designated "mbh-II-3", see NMR on page 6 of Exhibit 7). Subsequent notebook entries throughout the period of June 21-July 10, 2000 describe repeated attempts to synthesize additional amounts of the mbh-II-3 compound. After sufficient amounts of compound were obtained, notebook entries dated July 12-13, 2000 (pages 8-9, Exhibit 7) describe the bromination of mbh-II-3 and the successful synthesis of 2-bromo-3-hydroxy-4-methoxyacetophenone (therein designated "mbh-II-4"; see NMR of mbh-II-4 on page 11, Exhibit 7). Finally, in the last step for the synthesis of Intermediate #1, the TBS-protection of mbh-II-4 was described in series of notebook entries dated July 14-23, 2000 (page 10, Exhibit 7). Intermediate #1 (mbh-II-5) was successfully obtained on July 23, 2000 (see NMR of mbh-II-5 on page 12 of Exhibit 7). It turned out that a different methodology involving treatment of the appropriate silyl enol ether with bromine allowed for synthesis of the Intermediate #1, the TBS-protected bromoacetophenone, in satisfactory yield since the reaction could be performed in a single step (*i.e.,* the milder conditions did not displace the protecting group, and thus the product did not have to be reprotected).

15. After having obtained Intermediate #1, Hadimani and others proceeded to work on the synthesis of Intermediate #2 (TBS-protected 2-[3-hydroxy, 4-methoxyphenyl]-6-methoxyindole), according to the procedures outlined in Scheme III (see Exhibit 5, Page 5). Throughout the period from July 23-September 15, 2000, repeated attempts were made to synthesize Intermediate #2. However, problems of low yield caused delays in progress towards synthesis of the Compounds.

16. To constructively reduce my invention to practice, International PCT Application No. PCT/US00/25408 was filed on September 15, 2000, designating the United States of America along with other states (hereinafter the "PCT Application"). The PCT Application explicitly claimed Compounds XVII and XVIII (see Claims 17 and 19 respectively) and provided detailed descriptions for their synthesis (see Figures 12, 13, and 14).

17. On September 23rd, 2000, I assembled a six-person team of graduate and post-doctoral students ("Operation MAHJCK") to expedite the synthesis of the Compounds. Exhibit 8 provides notes taken during a meeting at which Operation MAHJCK was formed.

18. Exhibit 9 provides select pages from the notebook of Jimmy Kessler, one of the members of Operation MAHJCK. In a notebook entry dated September 28th, 2000, the synthesis of Intermediate #2 (therein designated "JK-I-61") is described using m-anisidine and Intermediate #1 (mbh-II-5) as starting materials. Successful synthesis of JK-I-61 was reported by NMR following purification by flash chromatography and recrystallization (see page 3, Exhibit 9). In a second notebook entry by Jimmy Kessler dated October 4, 2000 (see pages 4-6, Exhibit 9), the procedure for Friedel-Crafts acylation of JK-I-61 with 3,4,5-trimethoxy-benzoyl chloride was described and the successful synthesis of the TBS-protected derivative of Compound XVII ("JK-I-63") was achieved. Finally, as described in a notebook entry dated October 10, 2000 (see pages 7-10, Exhibit 9), JK-I-63 was deprotected to obtain Compound XVII (therein designated, "JK-I-65"). It appears that running the S_N2 reaction (between m-anisidine and Intermediate #1, see page 1, Exhibit 9) at a higher temperature (170°C) in dimethylaniline resulted in Intermediate #2 at a satisfactory yield, and in a single step.

19. Exhibit 10 provides select pages from the laboratory notebook of Mallinath Hadimani. In a series of notebook entries dated November 5-10, 2000, attempts to phosphorylate Compound XVII with dibenzyl phosphate (DBP) were recited. The DBP-protected derivative of Compound XVIII (therein designated, "mbh-III-4") was reported on November 10, 2000 (see

NMR on page 3, Exhibit 10). On November 11, 2000, the final step towards the synthesis of Compound XVIII, the deprotection of mbh-III-4, was initiated. After salting and recrystallization, the synthesis of Compound XVIII was reported on November 13, 2000; *see* the HPLC chromatogram that was run on this sample, which appears in Exhibit 10 of this Declaration. As there was some uncertainty at the time of these intensive experiments that the HPLC trace conclusively demonstrated that Compound XVIII had indeed been synthesized, a second synthesis was performed shortly thereafter. This second synthesis was confirmed as successful. However, an NMR run on the November 13, 2000 sample, which had been kept in refrigerated storage (page 7, Exhibit 10), confirmed that in fact Compound XVIII was made on November 13, 2000.

20. Taken together, the compounds and synthetic routes shown in the attached Exhibits and the information submitted in this Declaration demonstrate my conception of the present invention prior to February 16, 2000, and my diligence in reducing the invention to practice from the effective date of Pero date and up until September 15, 2000, the filing date of the subject application. Furthermore, the evidence submitted herein establish my diligence in reducing the invention to practice from the September 15, 2000 filing date until October 10, 2000 and November 13, 2000, on which respective dates Compounds XVII and XVIII were first successfully synthesized.

21. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Kevin G. Pinney

Inventor's Signature

March 5, 2004

Date

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